A. JUSTIFICATION OF NEED

A1. Requested instrument. The requested instrument is an integrated system comprising an AB Sciex 6500 triple quadrupole linear ion trap mass spectrometer with an electrospray and atmospheric pressure chemical ionization source, a Parker Balston Tri Gas Generator and a Shimadzu dual pump HPLC system with autosampler, column oven and controller. The system includes a workstation computer running AB Sciex Analyst Software for instrument control and data acquisition. This system comprises all components needed to conduct HPLC electrospray or atmospheric pressure chemical ionization tandem mass spectrometry measurements for structural analysis and quantitation of small molecules. Additional technical capabilities of the system that provide updated or new features that are not presently available to us are discussed below, summarized in the appendix and a detailed quotation from the vendor.

Although all of the major mass spectrometry instrument manufacturers (Agilent, Brucker, Waters, Thermo, Shimadzu) offer triple quadrupole instruments the ability of the ABSciex instrument to function with its third quadrupole operating as a linear ion trap is unique and provides enhanced capabilities to this hybrid instrument that include increased sensitivity and the ability to perform multistage fragmentation of ions that are selected and fragmented outside of the ion trap which is particularly important for analyte identification and elimination of interference in selected ion monitoring mode assays. As explained below, the PI of this proposal, Dr Morris and professional research staff associated with the Lexington VA Medical Center have extensive experience with AB Sciex multistage mass spectrometers that include 4000 series triple quadrupole linear ion trap instruments. We also have a significant investment in dedicated software for analysis of data generated by instruments from this manufacturer. Accordingly this manufacturer is our preferred choice. ABSciex offers 5500 and 6500 Q-Trap instruments. The primary difference between these instruments is that the 6500 instrument incorporates an improved ion source and a so called “Q-jet” ion guide that increases the efficiency of ion transfer into the high vacuum region of the instrument resulting in more effective ion containment, focusing and selection. These improvements allow the 6500 instrument to operate with a more sensitive higher energy detector. While sensitivity of these kinds of instruments is necessarily analyte specific, the increase in sensitivity of the 6500 instrument in comparison to the 4000 series instruments from the same manufacturer currently available to us for a representative analyte of interest (sphingosine 1 phosphate) is ~ 25-fold. This not only lowers the limit of detection for specific analytes but it also enables greater precision and sensitivity in selected ion monitoring mode measurements because dwell times for individual ion pairs can be shorter allowing collection of greater numbers of data points for more accurate peak integration. This is a particular advantage for many of our intended applications, notably measurements of multiple species of lipids within a particular class in a single HPLC MS/MS run. An additional feature of the requested instrument that is not available on the instruments currently available to us is that the polarity of the ion source can be rapidly (~20 ms) switched between positive and negative modes. Since many of the phospholipid analytes of interest to us are either anionic or cationic this capability will dramatically increase sample throughput by allowing us to monitor species that ionize in either positive or negative mode in the same LC MS/MS run.

A2. Purpose and relevance to Veterans Health. Obesity and associated chronic health conditions cause significant morbidity and negatively impact military readiness. Sixty-one to 83% of Department of Defense healthcare beneficiaries and 78% of Veterans are overweight or obese. Treatment of diseases associated with overweight and obesity is consistent with the priorities of the leadership of the Department of Veterans Affairs to promote personalized, proactive Veteran-driven care (VA/DOD Clinical Practice Guidelines for Screening and Management of Overweight and Obesity Version 2.0, 2014). Obesity is associated with known risk factors for cardiovascular disease including high blood pressure, elevated plasma triglycerides and cholesterol, alterations in the balance between HDL-associated “good cholesterol” and LDL-associated “bad cholesterol” and diabetes[1; 8]. However, obesity predicts cardiovascular disease risk even when these co-morbid conditions are not present[11]. A major research focus of investigators participating in this proposal is on investigating the concept that diet-dependent obesity and consequent hyperlipidemia result in changes in the lipid composition of circulating plasma components to initiate mechanisms that promote cardiovascular disease risk[7]. This is an important goal because although dietary restriction and exercise programs such as the VHA “Move” initiative can accomplish significant weight loss in Veterans, the majority of obese dieters fail to maintain their reduced weight. Given these challenges, a better understanding of the link between diet and cardiovascular disease risk that will result from completion of these studies might lead to improvements in healthful nutrition for obese or overweight veterans or identify targets for pharmacological intervention to mitigate cardiovascular disease risk associated with obesity in Veterans and active duty military personnel. Other users of the proposed instrument are studying the role of lipid metabolizing enzymes and bioactive lipids in disease

processes that include traumatic brain injury, the role of bone marrow-derived stem cells in heart repair following a heart attack and on the response of blood vessels to mechanical revascularization, all of which are similarly of high relevance to the mission and goals of the Veterans Health Administration.

A common theme of these studies is the concept that diet-dependent obesity and consequent hyperlipidemia and other pathological states result in changes in the lipid composition of circulating plasma components or tissues to initiate mechanisms that promote disease risk or disease processes. These studies are conducted using *in vitro* systems as well as preclinical (mainly rodent) models and human clinical studies. All of these projects require measurements of lipids of interest at low levels (pmol-fmol) usually in complex biological samples. HPLC electrospray ionization tandem mass spectrometry using multistage instruments is only analytical approach that can specifically detect and quantitate this class of molecules. The requested instrument is therefore required for completion of the aims of the VA funded research outlined above being conducted by the Major Users and also for research supported by NIH and other funds being conducted by VA-affiliated researchers in areas that are of high relevance to Veterans health.

A3. Inventory of instruments at the applicant's institution. The participating investigators are all part time VA employees with active VA research funding who concurrently hold appointments at the affiliated University of Kentucky College of Medicine. Accordingly the measurements required for their ongoing research programs are currently conducted using mass spectrometry instrumentation at the University of Kentucky which is paid for using VA research funds through a contract between the Lexington VA Medical Center and the University.

A3.1 Lexington VA Medical Center. No instrument of the type requested, and indeed no HPLC or mass spectrometry instrumentation of any type is currently available at the Lexington VA Medical Center.

A3.2 University of Kentucky. The Vice President for Research and the College of Medicine support four facilities containing a broad range of mass spectrometry instrumentation that can provide services to institutional investigators.

A3.2.1 University of Kentucky Mass Spectrometry Facility. The University of Kentucky Mass Spectrometry Facility operated by the Department of Chemistry provides basic mass spectrometry support to investigators in the broad areas of organic and materials chemistry which includes significant programs in biofuels research. It contains ion trap and time of flight instruments and a recently acquired Thermo Q-Exactive quadrupole orbitrap instrument. Involvement of this facility in NIH supported biomedical research is minimal and it is not available to provide services to VA funded researchers.

A3.2.2 Proteomics Core Laboratory. The proteomics core laboratory contains an AB Sciex Q-Star instrument, an ABSciex 5800 MALDI TOF/TOF instrument and a recently acquired Thermo LTQ Velos Obitrap with electron transfer dissociation capabilities. These instruments are used with chip based and nano flow HPLC systems for protein identification in complex mixtures or after gel excision. Some capabilities for analysis of post translational modifications are also offered. The laboratory contains a Thermo TSQ triple quadrupole mass spectrometer that is used for protein quantitation by measurement of peptides using selected ion monitoring approaches. While some proteomics services provided by this laboratory have been used for VA research, this facility cannot provide the capabilities needed for quantitation and structural analysis of small molecules by the Lexington VA Medical Center researchers.

A3.2.3 Resource Center for Stable Isotope Resolved Metabolomics. The mass spectrometry component of this center is primarily focused on using ultra high resolution instruments to monitor the distribution of stable isotope tracers among isotopologues of key metabolites. The two instruments used for this purpose are Thermo orbitrap fusion tribrid and Bruker Solarix XR FTMS mass spectrometers. These instruments are dedicated for use in an NIH U24 grant funded center which provides services to nation-wide investigators on a fee for service basis which includes personal and collaborative research programs investigating metabolic changes in cancer cells. These services do not encompass the kinds of measurements needed by the participating VA Medical Center researchers.

A3.2. 4 Small Molecule Mass Spectrometry Core Laboratory. The laboratory contains two ABSciex 4000 Q-Trap hybrid linear ion trap triple quadrupole mass spectrometers. These are operated with automated Shimadzu HPLC systems. The laboratory also contains an ABSciex 5600 Quadrupole time of flight mass spectrometer. This instrument can be operated with either an automated Shimadzu HPLC system or with an Eksigent microflow HPLC system that is configured for use with either capillary HPLC columns or to enable sample introduction by automated direct infusion. This microflow system operates with a low dispersion electrode insert that provides some of the sensitivity benefits of nano flow

electrospray ionization with increased robustness in comparison to glass emitter electrodes. This instrument can also be operated with an Advion Nanomate robotic nano electrospray ion source that is used for direct sample infusion and also to couple the instrument to a nano HPLC system. All of these instruments are serviced by Parker Balston Gas generators. The lab also contains a range of equipment for sample preparation including robotic liquid handling systems and solvent evaporators. This facility currently provides mass spectrometry services to the participating Lexington VA Medical Center investigators.

A3.3 Other instrumentation at the University of Kentucky and neighboring institutions. A number of individual investigator's personal laboratories and research centers at the University of Kentucky contain LC and GC coupled multistage mass spectrometers. These include the Center for Applied Energy Research, The Center for Tobacco Research and a USDA-affiliated animal feed testing laboratory associated with the College of Agriculture. None of these instruments can be made available for shared use. The only other major research university in the state, the University of Louisville is ~80 miles away. While this institution has instrumentation for mass spectrometry based proteomics its capabilities in small molecule mass spectrometry are relatively limited and in fact some of the University of Kentucky facilities outlined above routinely provide small molecule mass spectrometry services to several investigators at this institution. Other regional universities (Cincinnati, Ohio State and Vanderbilt) have extensive capabilities for small molecule mass spectrometry but use of their facilities for the quantity and scope of research we require would be unfeasible.

A4. Use of the University of Kentucky instrumentation is inappropriate for the current research needs of the Lexington VA Medical Center. The Small Molecule Mass spectrometry Laboratory in the University Of Kentucky College Of Medicine was developed by Dr Morris initially to support his own research program and that of collaborators (that include the other participating VA investigators). Between 2007 and 2011, all of the instrumentation was purchased in whole or in part using research funds generated through two NIH shared instrument grants, Recovery and Reinvestment Act supplements to personal grants and a Center for Biomedical Research Excellence (COBRE) grant. In 2013, this laboratory began functioning as an institution-wide core laboratory to provide defined services to local investigators on a standard recharge/fee for service basis under the Federal Cost Principles detailed in NOT-OD-13-053. This change was largely driven by growth in the research enterprise at the University of Kentucky and a need for services by investigators engaged in large NIH funded research programs that include the Center for Clinical and Translational Sciences, the National Cancer Institute designated Markey Cancer Center, The National Institute for Environmental Health Sciences supported University of Kentucky Superfund Research Center and the NIH supported Center for Research on Obesity and Cardiovascular Disease. The recharge arrangement recovers funds to support some effort for Dr Morris, two professional staff and the instrument service costs plus some shared costs for common equipment maintenance and shared supplies. Where necessary, assay specific costs are paid for by the individual users. The facility operates on a cost-neutral basis with service rates adjusted annually based on actual use (i.e. number of samples) and total operating costs. In FY 2014, the operating cost of this core was ~$200,000 and ~80% of this was recovered from service charges with the balance provided by the College of Medicine. While this arrangement works well to provide established defined services to institutional investigators it is not consistent with the needs of the Lexington VA Medical Center investigators for the logistical, technical and financial reasons outlined below.

1. The cost of services is prohibitive. Use of this instrumentation by VA investigators for VA funded research requires a contract between the Lexington VA Medical Center and the University Of Kentucky College Of Medicine at rate which include significant personnel costs. Although we have VA supported research staff that could be trained to use the instruments, the core arrangements do not accommodate direct use of the instruments by anyone other than the core personnel. The total cost of the current year contract is ~$40,000 to pay for ~1200 assays at $33/assay which both limits the number of assays we can conduct and makes it essentially impossible to use these instruments for discovery research. If we had our own instrument and could operate it ourselves, the same services could be provided at ~30% of the cost (see below) so we could run >3 times the number of assays for amount of VA funds that are currently being expended.

2. Instrument access is increasingly limited. The core instrumentation, particularly the triple quadrupole instruments, are now extremely heavily used with ~15,000 samples run in the past year. Given that typical run times for LC MS/MS assays are ~15-30 minutes and taking into account time needed for instrument calibration, tuning and cleaning, this means that almost all available instrument time is

accounted for. Accordingly, it has become increasingly difficult to get measurements made promptly or to have the instruments used to develop and validate new assays that are important and necessary for to achieve the goals of current and ongoing VA supported research.

3. Instruments cannot be readily used for discovery research/assay development. Because all use of the instruments has to be billed and paid for at pre-determined rates use of these instruments for assay development or discovery research (which can often require multiple LC MS/MS runs before a useful method is established) becomes very costly. As an example, a common interest of the VA funded researchers in in development of stable isotope based tracer methods to study lipid metabolism in live animals and humans. These studies would necessarily require development of methods to measure multiple mass isotopologues of lipid species of interest. The cost of setting up these studies on the University of Kentucky instruments at the current rates is prohibitive.

4. Reliability of older instruments is sub-optimal. The two triple quadrupole instruments available in the mass spectrometry core are ~7 years old and have been operated continuously since being put into service. Although these instruments are covered by maintenance contracts they have both experienced significant downtime particularly duet to failure of the high vacuum pumps in the past year.

5. The available instruments suffer from technical limitations that would be superseded by acquisition of updated state of the art instrumentation. The triple quadrupole instruments available are to us are ~10 year old technology and have significantly lower sensitivity in comparison to currently available state of the art instruments including the one we propose to acquire. This is due to improvements in the ion source, ion transmission optics and detector technologies that allow presently available instruments to operate at faster scan rates. As a result, a newer instrument would be able to detect and quantitate significantly lower levels of analytes (for example, the sensitivity for sphingosine 1 phosphate, a lipid of particular relevance to our interests, is ~25-fold higher). In addition to reducing the limits of detection and quantitation, this higher sensitivity results in greater precision and reproducibility because the shorter dwell times in selected ion monitoring (SIM) mode result in collection of a greater number of data points for more accurate peak integration. The sensitivity and mass resolution of the instrument we request is further increased by the hybrid function of its third quadrupole as a linear ion trap in tandem with precursor ion selection and fragmentation using the first quadrupole and collision cell. The high mass resolution of the linear ion trap and speed of the instrument in switching between ion transmission and ion trap modes enables high resolution SIM experiments for compound identification and elimination of interference. The instrument is also capable of rapid (20ms) polarity switching which enables simultaneous analysis of compounds that ionize in both positive and negative modes. All of these capabilities are not offered by the currently available instruments but would be of tremendous benefit to the research needs of the Lexington VA Medical Center researchers by increasing the speed, precision and sample throughput capacity.